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Scanning Probe Microscopy in the Study of Morphometric Changes and Physical Parameters of *Escherichia coli* Bacteria Under the Action of 2,4,6 - Trinitrotoluene

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Abstract: Using scanning probe microscopy (SPM) were registered charcteristic structural changes in *Escherichia coli* K12 cells under the influence of 2,4,6-trinitrotoluene (200 micrograms / ml). According to SPM data, changes were detected not only in the size of cells, but also in the structure of the cell wall. In the presence of TNT, the cell wall becomes less rough, flagellas and pils tend to be absent. However, the height of bacteria does not change. The data of force spectroscopy also showed differences in adhesion forces between the probe and the surface of the bacterial cell wall. In *Escherichia coli* K12 incubated with TNT there was a tendency to decrease an adhesive force compared with the control.

Key words: Scanning probe microscopy · *Escherichia coli* · Trinitrotoluene

INTRODUCTION

Nitroaromatic compounds are traditionally using as explosives and raw materials for the manufacture of dyes, synthetic polymers and solvents. They are also known as selectively acting drugs and pesticides. Most of them are very toxic and unnatural hardly decomposable compounds belonging to a number of environmentally hazardous substances. One of the representatives polynitroaromatic compounds is 2,4,6 - trinitrotoluene (TNT, trotyl) - a substance marked with severe toxic properties and its resistance to biodegradation. Toxic effects of TNT against bacteria is manifested not only in suppressing the growth of the culture, but also to change the morpho-physiological and physical properties of the cells. At high concentrations of TNT, there is a decrease in cell sizes, increase in granularity, as well as a decrease in the rate of glucose utilization and respiration. Transmembrane potential of the cells is changing that is indicates a distortion of transport functions of cell membrane [1, 2].

In this article, the possibility of scanning probe microscopy have allowed to analyze the changes described for the surface of bacteria on the micro - and nano-level, not only qualitatively but also quantitatively to aquire some numerical indicators.

MATERIALS AND METHODS

In this work, *E. coli* K12 strain was used. Cultivation was performed on a synthetic medium M9 supplemented with 0.4% glucose and 0.2% casein hydrolyzate. In experimental variant, TNT was added at a concentration of 200 mg / l. Before scanning, samples were washed with distilled water to remove the salts containing in the medium (buffer).

Visualization of the membrane surface of bacteria was carried out in air at room temperature and constant humidity in the semi-contact and contact modes on an atomic force microscope Solver P47H (production of "NT-MDT", Russia). We used standard silicon cantilevers (probes), the radius of curvature of the tip of which was not more than 10 nm. Scanning was performed with a resolution of 512x512 pixels. Adhesion force between the probe and the surface of the bacterial cell wall was measured by method of force spectroscopy.

RESULTS AND DISCUSSION

It has already been shown that under the influence of high concentrations of TNT cells with Gram negative morphotype of cell wall change their morphology. They are reduced in size, their granularity and refractive

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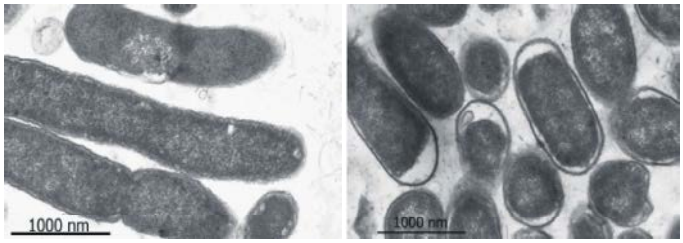

Fig. 1: Photomicrographs of *E. coli* K12 cell populations, obtained by the method of electron microscopy: a) - cells cultured without TNT b) - cells cultured with xenobiotic [4]. Bars correspond to 1 micron

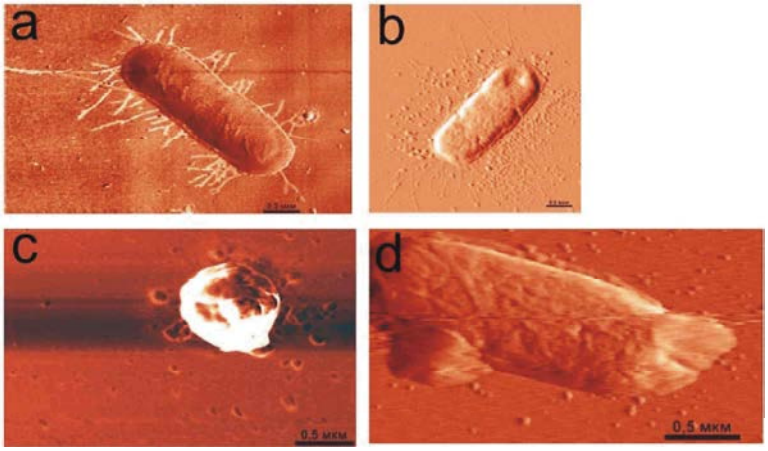

Fig. 2: AFM images of *E. coli* K12 cells obtained by scanning probe microscopy on glass: a) - the cell inoculum (zero point) (lateral force mode) b) 4 hour point - control cells (without TNT) (mode of phase contrast), c), d) - the cells in contact with xenobiotics. Bars correspond to 0.5 micron

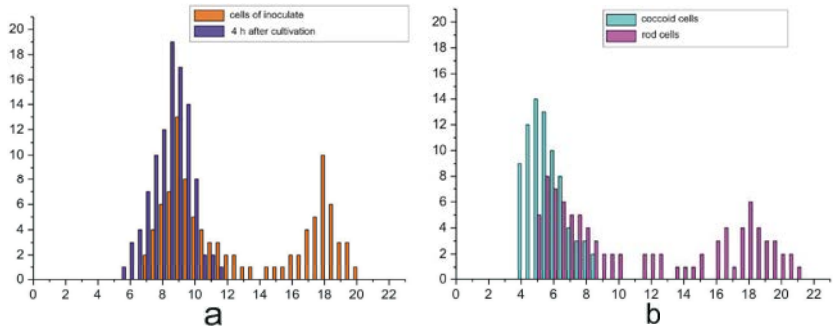

Fig. 3: Modified adhesion force between the probe and the surface of the cell wall of *E. coli* K12 bacteria: A - cells are not in contact with the xenobiotic (control) B - cells cultured with TNT during 4 hours. Axis Y – number of values, axis X – adhesion, nN

index is increased [3]. This change is typical at 4th hour of *Escherichia coli* (*E. coli*) cultivation. However, by 8th hour, the morphology of the test and control cells was not differred. Therefore, all subsequent work was performed on cells cultured for 4 hour in the presence of TNT (200 mg / l). As can be seen from Figure 1, the control cell population (a) cultivated without TNT, represented by single cells or chains of two cells [4]. The cells are rod-shaped, typical for *E. coli.* In populations incubated in the presence of TNT (b), cells are single, their size is much less than in controls. Along with rod-shaped cells, there were clearly visible cells in shape approaching to a spherical form. Contrary to the control cells, in an experimental variant, in rod-like,and round cells there was a significantly increased the periplasmic space.

A comparative analysis of the morphology of *E. coli* K12 bacteria incubated with TNT (experiment) and intact bacterial cells (control) enabled us to identify the following structural features of the structure of the surface layers (Figure 2). Along with intact *E. coli* K12 bacteria, we observed structures with a completely different morphometric characteristics: the shape and size of bacteria were changed (instead of rod cells with a length of 1.26±0.07 microns, bacteria have become rounded, with a radius of the order of 0.39±0.02 microns), the structure of the cell wall was less rough, flagellas and pils were disappeared, but the height of the bacteria has not changed (~0.22±0.02 microns).

Change of cell surface under the action of TNT can change the strength of adhesion force. Therefore, at the next phase of work, we measured the adhesion force between the tip of an atomic force microscope and the surface of the bacterial cell wall.

As seen in Figure 3, there were 2 maximums (9 and 18 nN) in the distribution of adhesion forces for the cells of inoculates. After 4 hours of culturing of *E. coli* K12 cells in a synthetic medium without TNT (control), there was a maximum value of the adhesion strength (9 nN).

Distribution of adhesion forces for cells in contact with TNT (experiment) differs depending on their morphology (Fig. 3B). For example, values of adhesion forces for rounded cells were reduced compared to control cells. Maximum of the distribution is shifted to 5 nN and an asymmetry of the distribution is observed. For rod-shaped cells, as well as for cells of an inoculum, there is a maximum 2 distribution. The first peak, located at low values of adhesion forces, equal in magnitude to a maximum for round cells (5.5 nN) and its distribution is also asymmetric. Second – a diffuse maximum of distribution is in the region of high values of adhesion forces. Its maximum value coincides with the second peak for the cells of inoculum (18 nN).

Therefore, toxic effects of TNT is manifested not only in reducing of the cell size, but also in changes of the surface structure (roughness decreases, pils and flagellas disappearence), as well as in reduction of adhesion forces between the probe and the surface of the bacterial cell wall. Reduction of adhesion forces between the tip and the surface of the cell wall may indicate an increase in its hydrophobicity [5]. This increase can be attributed to sorption of hydrophobic molecules of TNT at the cell surface, as well as with changes of protein-lipid interactions, which may be caused by the action of a xenobiotic or a reactive oxygen species (ROS) formed at the initial stages of its transformation.

CONCLUSION

Using scanning probe microscopy, it was found that under the influence of 2,4,6-trinitrotoluene (200 mg / l) the sizes of the *Escherichia coli* K12 cells were reduced and marked changes in the surface structure, which tend to reduce the surface roughness, the disappearance of flagella and pils, were detected. Under the influence of 2,4,6-trinitrotoluene (200 mg / l), there was tendency to decrease the adhesion forces between the tip and the surface of the cell wall of *Escherichia coli* K12, that may suggest about increase of its hydrophobicity.

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